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Hormonal factors and the risk of invasive ovarian cancer: a population-based case-control study

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Objective: To examine the influence of hormone-related factors on the risk of invasive epithelial ovarian cancer (ovarian cancer).

Design: Population-based case–control study using in-person interviews.

Setting: Academic department of preventive medicine.

Patient(s): Four hundred seventy-seven ovarian cancer patients and 660 controls.

Intervention(s): None.

Main Outcome Measure(s): Numbers of and ages at births, oral contraceptive use, and use of menopausal hormone therapy.

Result(s): Compared with nulliparous women, women whose only (last) birth was after age 35 years had an estimated 51% (95% confidence interval: 21%–70%) reduction in risk. If this birth occurred earlier, the reduction in risk was progressively less. Additional (earlier) births reduced the risk further. Oral contraceptive use also reduced risk. Increased body mass index increased risk, but this effect was confined to localized disease and is likely to be a diagnostic bias, as a consequence of other problems associated with being overweight and in itself having no etiological significance.

Conclusion(s): If the major protective effect of a late birth can be confirmed, our most challenging task will be to understand the mechanism to develop a chemoprevention approach to exploit this finding. (Fertil Steril® 2004;82:186–95. ©2004 by American Society for Reproductive Medicine.)

Key Words: Ovarian cancer, parity, estrogen therapy, oral contraceptives

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Epidemiological studies have consistently found that the risk of invasive epithelial ovarian cancer (ovarian cancer) is significantly decreased with increasing numbers of births, and Fathalla (1) proposed that the mechanism of this effect was the interruption of the tearing of the ovarian surface epithelium (OSE) with each ovulation (the *incessant-ovulation hypothesis*). The OSE is regarded by most, but not all, pathologists as the tissue of origin of ovarian cancer (2), and it was the increased cell division of the OSE that is involved in the repair of the surface after each ovulation that was presumed to be a major factor increasing ovarian cancer risk. The initial epidemiological findings of a significant protective effect of oral contraceptive (OC) use (3–5) appeared to provide further support for the incessant-ovulation hypothesis.

Since that time, a number of epidemiological and experimental findings have demonstrated that the incessant-ovulation hypothesis does not provide a comprehensive explanation of the etiology of ovarian cancer. These findings include a greater reduction in risk with first birth than with subsequent births (6), a greater reduction in risk with any birth than with a year of OC use (6), a continued rise in incidence with age after menopause (7), and a much lower ovarian cancer rate in "traditional" Japanese women than would be predicted by the hypothesis. The experimental finding of a stimulatory effect of estrogen (E) on benign ovarian tumor cells but an inhibitory effect of progesterone (8) suggests a possible unifying hypothesis for the etiology of ovarian cancer (8, 9).

We report here our investigations of these issues in a large population-based case–control study conducted in Los Angeles.

Epidemiological studies of epithelial ovarian cancer frequently include low malignant potential tumors with invasive tumors without distinction. We have not done this here, because molecular genetic studies suggest that such tumors are not part of a disease continuum but represent separate disease entities, and their age incidence is radically different (10).

MATERIALS AND METHODS

The study was approved by the institutional review board of the Keck School of Medicine of the University of Southern California. Informed consent was obtained from each patient and control before her interview.

Patient and Control Selection

Eligible patients were English-speaking non-Asian female residents of Los Angeles County who had histologically confirmed ovarian cancer or borderline (low malignant potential; LMP) ovarian tumors that were first diagnosed between 18 and 74 years of age, from October 1992 through October 1998. Patients and controls with previously diagnosed cancer (except nonmelanoma skin cancer) were not eligible. The reason for excluding such women is that treatment for a previous cancer may delay the appearance of an ovarian cancer or alternatively lead to its earlier diagnosis. The latter phenomenon was clearly seen in our case identification, in which 31 ovarian tumors were diagnosed during the workup of another cancer (most commonly endometrial cancer); these cases are not included in the numbers given in the next paragraph. The cases were identified by the Cancer Surveillance Program, the cancer registry covering all residents of Los Angeles County. Patients with borderline endometrioid tumors were excluded from the study because this subtype is classified as a benign tumor by the International Agency for Research on Cancer and is not ascertained by the Cancer Surveillance Program or other certified North American cancer registries.

A total of 1,442 patients meeting the pathological case definition were identified by the Cancer Surveillance Program. We identified 139 of these patients as not English speaking, leaving 1,303 eligible patients. Of these, 291 patients had died or were too ill to be interviewed by the time that we contacted their physicians; the patients' physicians refused permission to contact an additional 65 patients; 31 patients were no longer residents of Los Angeles County and had moved too far away to be interviewed in person; 66 patients could not be located; and 173 patients declined to be interviewed. Interviews were conducted with 677 patients (52% of all patients and 80% of patients approached): 491 of these were classified as having ovarian cancer, and 186, as having LMP tumors.

Controls were English-speaking non-Asian women with at least one intact ovary, individually matched with patients on race and ethnicity (African-American, Latina, non-Latina White) and date of birth (±3 years). Initially, a neighborhood control was sought by one of our staff who physically canvassed the neighborhood using a systematic algorithm based on the address of the patient. If the first eligible match refused to participate, the second eligible match in the sequence was asked, and so on. Letters were left when no one was home, and follow-up by mail, telephone, and further visits to the neighborhood continued until either an eligible control agreed to be interviewed or 150 housing units had been screened. For patients aged >65 years, if no willing control could be found in the first 100 housing units, a control was simultaneously sought among a random sample of female residents of Los Angeles County aged >65 years who were provided to us by the Health Care Financing Administration. The Health Care Financing Administration control was matched on the patient's zip code, race and ethnicity, and date of birth (closest to that of the patient).

When the control had an ovary-sparing hysterectomy before her reference date (12 months before the diagnosis of her matching patient) but was matched with a patient who had not had a hysterectomy, a second control who had not had a hysterectomy by that date was sought. Altogether, 664 controls were successfully interviewed by the closing date of the study. The first eligible match was interviewed for 70% of the patients, and the second match, for another 21%. At the termination of the study, 530 of the interviewed patients were matched with at least 1 interviewed control, 136 of the interviewed patients had no matching interviewed control, and 104 interviewed controls were matches for patients who had not been successfully interviewed or turned out to be ineligible. In total, 30% of eligible controls identified declined to be interviewed (the same proportion as that of first eligible controls declining to be interviewed).

This report is confined to ovarian cancer patients, but use is made of all interviewed controls.

Risk Factor Assessment: Questionnaires

Each patient was interviewed in person by using a comprehensive questionnaire covering medical, gynecological, reproductive, and certain aspects of personal lifestyle history, up to 12 months before her diagnosis date (her reference date). Calendars were used to chart major life events and reproductive and contraceptive histories. Each control was interviewed in the same manner, with the pseudo-reference date taken as the reference date of her matched patient.

Data Analysis

Statistical analyses were conducted by standard statistical methods (11) including multivariate logistic regression (EPILOG statistical package program; EpiCenter Software, Pasadena, CA). Although the study was designed as a matched case–control study, a significant number of patients did not

have a matched control, and we wished to include the controls of the LMP patients, so a multivariate unconditional logistic regression analysis approach was adopted. Adjustments were made for three race and ethnicity groups (African-Americans, Latinas, non-Latina Whites), 5-year age groups, four levels of socioeconomic status (SES) according to census tract of residence at the time of diagnosis (12), and four levels of education.

All the reported risk estimates were in addition adjusted for the following factors: family history of ovarian cancer (mother or sister; yes/no), tubal ligation (yes/no), use of genital area talc (yes/no), usual body mass index (BMI) during 5 years before reference date (kilograms per meter square; categorical variable), nulliparity (yes/no), age at last (term) birth (ALB; ALB at 35+ years and per 5-year group before 35+ years; continuous), number of additional births (i.e., zero, or total births minus 1, whichever is the greater; continuous variable), number of incomplete pregnancies (continuous), duration of OC use (months; continuous), type of menopause (premenopausal vs. natural vs. surgical), age at natural menopause (5-year age groups; continuous), age at surgical menopause (5-year age groups; continuous), and duration of menopausal hormone therapy use (E–progestin therapy [EPT] use by hysterectomized women, EPT use by naturally menopausal women, E therapy [ET] use by hysterectomized women, and ET use by naturally menopausal women; all continuous). All statistical significance values (*P* values) quoted are two sided and are standard χ^2 analyses based on differences in log likelihoods (11).

Women undergoing a hysterectomy without a bilateral oophorectomy (simple hysterectomy) before menopause were classified as having a surgical menopause. For naturally menopausal women, age at menopause was estimated as follows: for a woman taking OCs, age at menopause was taken as the end of the period of OC use, if no "natural" menstruation occurred thereafter. Natural menstruation was taken to mean menstruating and not using OCs or menopausal hormone therapy (HT) at the time. For a woman taking HT to within 3 months before her reported age at last menstrual period, we set her age of menopause at the date on which she began HT use, with the rationale that HT use was started because of menopausal symptoms.

We elsewhere have given the justification for this approach to setting age at menopause (13). Essentially, age at last menstrual period cannot be used to uniformly estimate age at menopause because women who use sequential EPT usually continue to have monthly menstrual periods, irrespective of their ovarian function, and women on ET and continuous-combined EPT can rarely distinguish breakthrough bleeding from ovarian function–determined menses.

The E component of OCs was considered high if the dose of ethinyl estradiol was >35 μg or if the dose of mestranol was >70 μg. The progestin component was considered high if the dose was equivalent to ≥0.30 mg of DL-norgestrel. The conversion to assumed equivalent DL-norgestrel doses was done based on the results of studies of Greenblatt, as described in Dickey and Stone (14).

TABLE 1

Demographic characteristics of ovarian cancer patients and controls.

	Controls		Patients	
Characteristics	n	%	n	%
Race/ethnicity				
African American	50	7.6	44	9.2
Latina	92	13.9	61	12.8
White	518	78.5	372	78.0
Total	660		477	
Age (y)				
<35	75	11.4	20	4.2
35–44	98	14.8	62	13.0
45–54	203	30.8	140	29.4
55–64	138	20.9	144	30.2
65+	146	22.1	111	23.2
Socioeconomic status				
1 (high)	242	36.7	176	36.9
2	188	28.5	117	24.5
3	100	15.2	71	14.9
4 (low)	130	19.7	113	23.7
Education				
<High school	41	6.2	44	9.2
High school	174	26.4	154	32.3
Some further training	340	51.5	229	48.0
College graduate	105	15.9	50	10.5

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RESULTS

Eleven patients were found to have had a previous cancer at interview and were thus deemed ineligible. Three ovarian cancer patients and two controls were eliminated because of reported age of <35 years at natural menopause, and two additional controls were excluded because of contradictory data. The race and ethnicity, age, SES, and education of the remaining patients and controls are shown in Table 1. The patients are older than the controls; this is because LMP tumors occur at a younger age than do invasive tumors, and the controls were matched to both LMP tumor patients and ovarian cancer patients.

Analysis of family history (in mother or sister) of ovarian cancer, tubal ligation, and use of genital area talc showed the well-known effects: odds ratios (ORs) were 3.78 for family history, 0.82 for tubal ligation, and 1.60 for talc (Table 2). Although the reduced risk associated with tubal ligation was not statistically significant in these data, all three factors have been included in our statistical model because they are well-established risk factors. Table 2 also shows that risk was increased in women with BMI (weight in kg/height in

TABLE 2

Analysis of some known risk factors for ovarian cancer.

Risk factor	Controls	Patients	Adjusted OR[a]	95% CI	Statistical significance χ^2 (df)	Statistical significance *P* value
Family history of ovarian cancer						
No	648	448	1.00			
Yes	12	29	3.78	1.83–7.80	14.33 (1)	.0002
Tubal ligation						
No	579	431	1.00			
Yes	81	46	0.82	0.53–1.26	0.86 (1)	.35
Genital area talc						
No	544	349	1.00			
Yes	116	128	1.60	1.18–2.18	9.05 (1)	.0026
BMI (kg/m^2)[b]						
<25	397	261	1.00			
25–29	165	120	0.97	0.71–1.33		
30–34	60	56	1.29	0.83–1.99		
35+	38	40	1.46	0.87–2.44	3.44 (3)	.32

Note: CI = confidence interval; df = degrees of freedom; ALB = age at last (term) birth; EPTH = EPT used by hysterectomized women; EPTM = EPT used by naturally menopausal women; ETH = ET used by hysterectomized women; ETM = ET used by naturally menopausal women; SES = socioeconomic status.

[a] Adjusted for ethnicity (3 groups), age (9), SES (4), education (4), family history of ovarian cancer (2), tubal ligation (2), use of genital area talc (2), BMI (4), nulliparity (2), ALB (at 35+ y, per 5-y group before 35+ y), number of additional births (continuous), number of incomplete pregnancies (continuous), OC use (continuous), menopausal status (3), age at natural menopause (5-y age groups, continuous), age at surgical menopause (5-y age groups, continuous), ETM (continuous), ETH (continuous), EPTM (continuous), and EPTH (continuous).

[b] Calculated with usual weight during 5 y before reference date.

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meters squared) of ≥30 kg/m^2; although this result was also not statistically significant, it too has been included in our final statistical model because there is substantial evidence for this effect (15).

Women who had four or more (term) births had a 64% reduced risk of ovarian cancer compared with nulliparous women (Table 3). Risk declined with increasing numbers of births (OR = 0.83 per birth; $\chi^2_1 = 17.59$; $P<.0001$). The effect of the first birth was greater than that of succeeding births (OR = 0.68 compared with OR = 0.86), although this difference was not statistically significant.

The OR associated with ALB are also shown in Table 3. There is good evidence that the later the ALB, the lower the risk. Fitting nulliparity (nulliparous vs. parous) and ALB (among parous women) gave $\chi^2_4 = 21.15$ ($P=.0003$); fitting nulliparity and ALB as a continuous variable gave $\chi^2_2 = 19.93$ ($P<.0001$). Each additional birth reduced risk by 10% ($\chi^2_1 = 3.10$; $P=.078$). We observed a strong positive relationship between late age at first birth and late ALB and were not able to clearly distinguish between them. This was so even when we restricted analysis to women with two or more children. We kept ALB in the analysis because the result was clearer (steady effect in most subgroups we studied).

As we have seen, the effect of fitting additional births after fitting ALB was not statistically significant, but increasing numbers of incomplete pregnancies was associated with a statistically significant reduced risk of ovarian cancer ($\chi^2_1 = 4.29$; $P=.038$; Table 3), and both variables (additional births and incomplete pregnancies) have been retained in the statistical model. There was no effect of age at incomplete pregnancy, and, in particular, replacing ALB with age at last pregnancy led to a reduction in the fit of the model to the data. Fitting separate terms for spontaneous and induced abortions showed a stronger protective effect of reported induced abortions, but the difference was not statistically significant.

Breast-feeding the last baby was associated with a 23% reduction in risk, but the result was not statistically significant, and breast-feeding other babies was not associated with any reduction risk. We did not include breast-feeding in our final statistical model.

Oral contraceptive use was associated with a statistically significant protective effect (Table 4). There was a 5.8% protective effect per year of OC use ($\chi^2_1 = 16.72$; $P<.0001$). There was no differential effect of age at OC use; dividing the age at use into four categories (<25 years, 25–29 years, 30–34 years, and 35+ years) was associated with no pattern of effect (difference from single term, $\chi^2_3 = 5.54$; $P=.14$). Categorizing OCs into four groups based on their E and progestin content showed no significant differences ($\chi^2_3 =$

TABLE 3

Complete and incomplete pregnancies and risk of ovarian cancer.

Variable	Controls	Patients	Adjusted OR[a]	95% CI	Statistical significance χ^2 (df)	Statistical significance *P* value
Births						
0	139	120	1.00			
1	103	65	0.62	0.40–0.96[b]		
2	174	127	0.62	0.42–0.90[b]		
3	124	90	0.55	0.36–0.84[b]		
4+	120	75	0.36	0.22–0.57[b]	19.46 (4)	.0006
Per birth			0.83	0.76–0.91[b]	17.59 (1)	<.0001
First birth			0.68	0.48–0.97[c]		
Per additional birth			0.86	0.78–0.96[c]	18.93 (2)	<.0001
ALB (y)						
Nulliparous	139	120	1.00			
<25	92	94	0.84	0.55–1.29[d]		
25–29	170	110	0.55	0.37–0.81[d]		
30–24	158	100	0.50	0.33–0.74[d]		
35+	101	53	0.42	0.26–0.67[d]	21.15 (4)	.0003
ALB at 35+			0.49	0.30–0.79		
Per 5-y group before age 35 y			1.18	1.01–1.38	19.93 (2)	<.0001
Per additional birth			0.90	0.80–1.01	3.10 (1)	.078
Incomplete pregnancies						
0	402	300	1.00			
1	154	98	0.86	0.62–1.17		
2	58	40	0.93	0.59–1.48		
3	23	12	0.77	0.36–1.66		
4+	22	7	0.36	0.14–0.92	5.91 (4)	.21
Per incomplete pregnancy			0.88	0.77–0.99	4.29 (1)	.038

Note: CI = confidence interval; df = degrees of freedom; ALB = age at last (term) birth; EPTH = EPT used by hysterectomized women; EPTM = EPT used by naturally menopausal women; ETH = ET used by hysterectomized women; ETM = ET used by naturally menopausal women; SES = socioeconomic status.

[a] Adjusted for ethnicity (3 groups), age (9), SES (4), education (4), family history of ovarian cancer (2), tubal ligation (2), use of genital area talc (2), BMI (4), nulliparity (2), ALB (at 35+ y, per 5-y group before 35+ y), number of additional births (continuous), number of incomplete pregnancies (continuous), OC use (continuous), menopausal status (3), age at natural menopause (5-y age groups, continuous), age at surgical menopause (5-y age groups, continuous), ETM (continuous), ETH (continuous), EPTM (continuous), and EPTH (continuous).

[b] Adjustment variables excluded: nulliparity, ALB, and additional births.

[c] Adjustment variables excluded: nulliparity and ALB.

[d] Adjustment variables excluded: nulliparity and additional births.

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4.90; *P*=.18), although the reduction in risk was greater with high-progestin OCs and was most marked in the low E with high-progestin OCs (Table 4).

No differences were observed between patients and controls in the occurrence of difficulties in getting pregnant or in the use of fertility drugs (data not shown).

No effect of age at menarche was seen (data not shown).

There was a trend for later age at natural menopause to be associated with increased risk (OR = 1.19 per 5 years later; Table 5), and there was also a trend for later age at hysterectomy to be associated with an increased risk of ovarian cancer (OR = 1.10 per 5 years later; Table 5), but the results were not statistically significant.

Menopausal ET use in naturally postmenopausal women was associated with an increase in risk of ovarian cancer (OR = 1.16 per 5 years of use), but the result was not statistically significant (Table 5). Estrogen-progestin therapy use was not associated with any increase in risk. The results for ET use in hysterectomized women were very similar (OR = 1.11 per 5 years of use) to those seen with naturally menopausal women (Table 5). The numbers of EPT users in hysterectomized women were too small for meaningful analysis.

Stage at diagnosis was recorded by the cancer registry for 464 (97.3%) of the 477 patients: 100 (21.6%) of the patients were classified by the Surveillance, Epidemiology, and End Results registry as localized (confined entirely to the organ of origin), and 364 (78.4%), as having regional or distant

TABLE 4

Oral contraceptive (OC) use and risk of ovarian cancer.

Variable	Controls	Patients	Adjusted OR[a]	95% CI	Statistical significance χ^2 (df)	Statistical significance *P* value
OC use (y)						
Never	245	222	1.00			
<5	246	169	1.00	0.72–1.39		
5–9	90	51	0.72	0.46–1.13		
10+	79	35	0.48	0.29–0.78		
Per y of use			0.94	0.91–0.97	16.72 (1)	<.0001
OC formulation						
High E + high P	40	20	0.88	0.81–0.97		
High E + low P	70	41	0.94	0.88–1.00		
Low E + high P	10	4	0.66	0.36–1.21		
Low E + low P	203	132	0.95	0.92–0.99		
Unknown	151	100	0.96	0.90–1.02	21.95 (5)	.0005

Note: CI = confidence interval; df = degrees of freedom; ALB = age at last (term) birth; EPTH = EPT used by hysterectomized women; EPTM = EPT used by naturally menopausal women; ETH = ET used by hysterectomized women; ETM = ET used by naturally menopausal women; SES = socioeconomic status.

[a] Adjusted for ethnicity (3 groups), age (9), SES (4), education (4), family history of ovarian cancer (2), tubal ligation (2), use of genital area talc (2), BMI (4), nulliparity (2), ALB (at 35+ y, per 5-y group before 35+ y), number of additional births (continuous), number of incomplete pregnancies (continuous), OC use (continuous), menopausal status (3), age at natural menopause (5-y age groups, continuous), age at surgical menopause (5-y age groups, continuous), ETM (continuous), ETH (continuous), EPTM (continuous), and EPTH (continuous).

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(more extensive) disease. Analyzing the two groups of patients separately showed important differences in the estimates of the effects of BMI. The odds ratios increased sharply over the four increasing BMI categories (<25, 25–29, 30–34, and 35+) for localized disease, from 1.00 to 1.42 to 2.66 and finally to 3.43 for BMI of 35+ (χ^2_3 = 11.64; *P*=.0087). There was only a very weak, not statistically significant relationship between BMI and risk of regional or distant disease; the odds ratios over the same increasing BMI categories were 1.00, 0.89, 1.07, and 1.16. The effect of ET was also more evident for localized disease: for localized disease, the ORs for 5 years of use were 1.51 and 1.22 for natural menopause and hysterectomized women, respectively, whereas for regional or distant disease, the ORs were 1.11 and 1.10, respectively.

The patients were histologically classified as follows: 324 as serous, 92 as endometrioid (including clear cell), 42 as mucinous and 19 as other (or unknown). There was a marked relationship between stage and histology: for serous tumors, 12% were localized; for endometrioid, 42%; and for mucinous, 55%. With endometrioid tumors, a last birth at age 35+ years reduced risk by 80%, whereas for serous tumors, the reduction in risk was only 36% (*P* value for difference in effect, .039). There were too few data to make further meaningful comparisons.

DISCUSSION

The most striking finding in this study was a possible major effect of ALB; the later the ALB, the lower the risk of ovarian cancer. Compared with a nulliparous woman, the overall risk of ovarian cancer in a woman with a last birth after age 35 years was reduced by 58%, with a reduction of 51% due solely to the last birth. A last birth before age 25 years was associated with only a 16% reduced risk of ovarian cancer; even after adjusting for numbers of births, this only increased to 20%. Additional births (before the last) reduced risk further by some 10% per birth.

These results for ALB must, however, be regarded as somewhat tentative for the following reason: fitting numbers of births as a single linear term was significantly associated with decreasing ovarian cancer risk (Table 3; χ^2_1 = 17.59). Including ALB with numbers of births improved this fit (χ^2_3 = 23.03), but this improvement is not quite statistically significant (χ^2_2 = 5.44, *P*=.066). If, based on the results of this and previous studies, we regard the effect of first birth as being greater than that of subsequent births, the fit to the data is measured by χ^2_2 = 18.93 (Table 3). In comparison to this, fitting ALB in addition to number of births is just statistically significant (χ^2_1 = 4.10, *P*=.043).

Age at last pregnancy provided a less good fit to our data than ALB.

Oral contraceptive use reduced ovarian cancer risk by approximately 6% per year of use, a smaller effect than seen with births. Age at last use of OCs and whether the use occurred before or after last pregnancy had no differential effect on risk. There was no evidence of an increased effect of OC use with increasing age at use. There was a greater protective effect with OCs containing a high-dose progestin, especially if the high-dose progestin was combined with a low dose of ethinyl estradiol, but these results were not statistically significant.

Although the differences were not statistically significant, both additional births and OC use were associated with a greater protective effect against ovarian cancers diagnosed at younger ages; reductions in risk in cancer diagnosed at age <55 years, at 55–64 years, and at 65+ years were per additional birth and per year of OC use (respectively): 0.79 and 0.92, 0.82 and 0.96, and 1.05 and 0.98. This effect was not seen with ALB.

Earlier ages at both natural and surgical menopause were associated with decreased risks of ovarian cancer. Postmenopausal ET was found to increase ovarian cancer risk by approximately 11%–16% per 5 years of use in naturally and surgically menopausal women. The effect was smaller in regional or distant disease. Estrogen-progestin therapy use was effectively confined to naturally postmenopausal

TABLE 5

Menopause and use of menopausal ET and EPT and risk of ovarian cancer.

Variable	Controls	Patients	Adjusted OR[a]	95% CI	Statistical significance χ^2 (df)	Statistical significance *P* value
Natural menopause						
Age at natural menopause (y)						
<45	44	31	1.00			
45–49	94	74	1.29	0.71–2.35		
50–54	129	113	1.67	0.94–2.99		
≥55	32	23	1.44	0.65–3.17		
Per 5 y			1.19	0.95–1.49	2.29 (1)	.13
ET (mo)						
0–12	263	215	1.00			
13–60	19	11	0.71	0.32–1.61		
61+	17	15	1.36	0.62–2.96		
Per 5 y			1.16	0.92–1.48	1.53 (1)	.22
EPT (mo)						
0–12	188	175	1.00			
13–60	54	26	0.60	0.34–1.05		
61+	57	40	0.90	0.55–1.48		
Per 5 y			0.97	0.77–1.23	0.05 (1)	.82
Surgical menopause						
Age at hysterectomy (y)						
<35	21	25	1.00			
35–39	18	23	1.30	0.52–3.22		
40–44	23	31	1.23	0.52–2.91		
≥45	8	12	1.38	0.44–4.32		
Per 5 y			1.10	0.79–1.53	0.30 (1)	.59
ET (mo)						
0–12	36	40	1.00			
13–60	12	17	1.12	0.44–2.86		
61+	22	34	1.56	0.72–3.41		
Per 5 y			1.11	0.92–1.35	1.19 (1)	.28
EPT (mo)						
0–12	67	84	1.00			
13–60	1	4	5.04	0.50–51.22		
61+	2	3	1.13	0.15–8.31		
Per 5 y			1.30	0.63–2.67	0.60 (1)	.44

Note: CI = confidence interval; df = degrees of freedom; EPT = E-progestin therapy; SES = socioeconomic status; ALB = age at last (term) birth; EPTH = EPT used by hysterectomized women; EPTM = EPT used by naturally menopausal women; ETH = ET used by hysterectomized women; ETM = ET used by naturally menopausal women

[a] Adjusted for ethnicity (3 groups), age (9), SES (4), education (4), family history of ovarian cancer (2), tubal ligation (2), use of genital area talc (2), BMI (4), nulliparity (2), ALB (at 35+ y, per 5-y group before 35+ y), number of additional births (continuous), number of incomplete pregnancies (continuous), OC use (continuous), menopausal status (3), age at natural menopause (5-y age groups, continuous), age at surgical menopause (5-y age groups, continuous), ETM (continuous), ETH (continuous), EPTM (continuous), and EPTH (continuous).

Pike. Hormonal factors and ovarian cancer risk. Fertil Steril 2004.

women, and in these women, no effect of EPT use was evident.

An increased BMI was significantly associated with increased risk of localized ovarian cancer, but there was only a slight association with regional or distant disease. This suggests to us that it is likely that increasing BMI may bring a woman more frequently to medical attention and that localized disease is then found incidentally during the workup of other problems (such as irregular bleeding). At present, we do not have adequate data to investigate this further.

The above results were generally seen with the different histologic varieties of ovarian cancer. The difference that was suggestive of a real effect was the observation that endometrioid (and clear cell) tumors were more profoundly reduced with ALB at age 35+ years (80% reduction, compared with 36% for serous tumors); this needs confirmation.

We investigated to what extent some of these results could be due to the fact that we only managed to interview 52% of the potential patients (80% of those approached). There were differences between the interviewed and noninterviewed patients on a number of factors: those interviewed were younger (age <45 years: 17.2% vs. 14.5%; 45–54 years: 29.4% vs. 19.2%; 55+ years: 53.4% vs. 66.3%), were of higher SES (SES 1: 36.9% vs. 20.1%; SES 2–3: 39.4% vs. 47.1%; SES 4: 23.7% vs. 32.8%), and had a different ethnic mix (African-American: 9.2% vs. 17.7%; Latina: 12.8% vs. 8.3%; White: 78.0% vs. 74.0%). These differences should not matter because all analyses adjusted for age, SES, and ethnicity. However, the interviewed patients were biased toward "earlier" stage at diagnosis (localized: 22% vs. 14%), and as we have seen, this does affect the overall relationship between BMI and ovarian cancer risk because it is only the localized patients that show any such relationship. As we discussed above, this observed effect of BMI requires further investigation.

How do these results fit in with previous studies? A profound effect of ALB in close agreement with our results was seen in the recently published study of Whiteman et al. (16). An effect of age at births was first noted in the four US population-based case–control studies analyzed by Whittemore et al. (6); those investigators observed a reduced risk of ovarian cancer with late age at first birth. As noted above, in the study reported here, we observed a strong positive relationship between late age at first birth and late ALB, and the result found by Whittemore et al. (6) is thus likely to be supportive of an effect of late ALB. Titus-Ernstoff et al. (17) also observed a trend of increased protective effect with late ALB.

No effect of ALB was seen in a Canadian population-based case–control study (18) or in a Swedish population-based case–control study (19). We have no explanation for these apparently contradictory results. It may be that the high proportion (40%) of immigrants in the Canadian study, which was not adjusted for in the analysis, produced their result. The Swedish study used a mailed questionnaire: were the inherent limitations in such an approach a contributing factor to their results? No other published studies had covariate data available (in particular, OC data) to adjust their estimates of the effects of age at births, and thus this variable is not interpretable in other studies.

Oral contraceptive use has been consistently found to be associated with a reduced risk of ovarian cancer (3–6, 9, 18, 20, 21). The results from cohort and population-based case–control studies suggest a reduction in risk of around 8%, with a slightly reduced risk with longer time since last use. A recent reanalysis of the Cancer and Steroid Hormone Study (9) suggested that OC formulations with a "high-dose/potency" progestin may be associated with a greater reduction in risk than those with a "low-dose/potency" progestin. We observed this effect, but our results were not statistically significant, and Ness et al. (20) did not observe such an effect. Further data are needed to adequately evaluate this progestin dose issue. No current commonly prescribed OCs contain a high-dose progestin.

Hysterectomy consistently has been found to be a significant protective factor against ovarian cancer (see, for example, Irwin et al. [22], Hankinson et al. [23], and Green et al. [24]). We did not fully address this issue in our study because only women who were sure that they had not had a bilateral oophorectomy at the time of their hysterectomy were included as controls. This meant that we will have excluded some women as controls who did not have a bilateral oophorectomy at the time of their hysterectomy but who were not sure that this was the case: this leads to a reduction in the estimate of any reduction in risk associated with hysterectomy, and we considered hysterectomy to be a variable that needed to be adjusted for in all our analyses, not one for which we could obtain a valid estimate of protective effect. It was not practical for us to avoid this problem by obtaining operation records for all hysterectomized potential controls.

We did, however, see a much greater protective effect from an earlier rather than a later hysterectomy, and this strongly suggests a real age-dependent protective effect of hysterectomy. This age-dependent protective effect of hysterectomy has been consistently found and, in particular, was found in the Nurses Health Study prospective cohort (23), in which recall bias is not an issue: in this study, a 52% reduction in risk was found with hysterectomy before age 45 years, compared with a 24% reduction with hysterectomy after this age.

The age incidence of ovarian cancer shows a marked slowing of the rate of increase around age 50 years (even after allowing for the effect of oophorectomies, hysterectomies, and tubal ligations by studying this phenomenon in countries and in time periods in which these operations were uncommon); this clearly suggests a protective effect of menopause (7). However, epidemiologic studies have not found this effect consistently. Although an increased risk of ovarian cancer in association with late age at menopause has been reported in a few studies, no association between age at natural menopause has been reported in most studies (see Schildkraut et al. [25] and references therein).

Why an effect of age at menopause is not consistently found is difficult to explain. It is possible that the difficulty may be because the early stages of ovarian cancer affect ovarian function and cause an early menopause. If this is so, then a more clear effect of age at menopause should be seen if one restricts attention to ovarian cancer patients diagnosed after 65 years of age, say. Such analyses have not been carried out and would be most informative. The lack of an effect of early menarche can, of course, be readily explained on an unopposed-E hypothesis because early menarche is associated with a more rapid onset of regular (ovulating)

cycles than a late menarche, so that amount of exposure to unopposed E may well be unaffected by age at menarche.

The current epidemiologic evidence obtained from cohort and population-based case–control studies with data on formulation and duration of use of menopausal hormone therapy (HT; 26–36) suggests that therapy with unopposed E increases ovarian cancer risk. Our meta-analysis of these studies finds an OR of approximately 24% per 5 years of use, with a 95% confidence interval of 13%–37% (37). The estimated overall risk associated with EPT use is smaller (13% per 5 years of use) and is not statistically significant. Some of this excess risk may be a diagnostic bias because we found that the ET effect was smaller when we restricted attention to regional or distant disease. A joint careful analysis of the published studies is needed to help clarify this issue.

17β-Estradiol has been found to increase ovarian cancer cell and ovarian cystadenoma cell proliferation in vitro (8), so the mechanism of any ET effect may be by direct stimulation of growth of the relevant premalignant or early malignant cells. Progesterone reduces cell proliferation under the same in vitro conditions, so one might predict that EPT would have a lesser effect on risk of ovarian cancer than ET. The relevance of these in vitro experiments is, however, open to some question because the doses of steroids used are much greater than those achieved with HT.

We saw a positive correlation between BMI and ovarian cancer risk in this study, and a recent systematic review (15) found a consistent positive association between body size and ovarian cancer risk in 11 population-based case–control studies and 5 cohort studies. Since that review, results from 2 other population-based case–control studies (38, 39) and 5 cohort studies (40–44) have been published. Two of these studies (41, 44) did not find such an effect; all the other studies did. The increase in risk between the upper and lower quartiles of BMI is around 40%. As we discussed above, we only saw this relationship with localized disease, and further investigation of this relationship is needed to determine its etiologic significance.

If BMI is related to ovarian cancer risk only incidentally, through increasing BMI being related to increased contact with the medical system, then this would cast some doubt on the increased incidence that is seen with ET use because BMI is associated with increased postmenopausal E levels and decreased sex hormone–binding globulin. One would therefore expect to see a clear association with BMI if the ET result was true.

Oral contraceptives block ovulation and the subsequent repair of the ovarian surface (the purported cell of origin of ovarian cancers). Rodriguez et al. (45, 46) have proposed, based on long-term studies in macaques of extended exposure to OCs or the individual components of OCs, that it is a direct action of progestins on OSE that provides the protection from OCs against ovarian cancer. In these studies, the progestin component of OCs, given alone without the E component, showed an increased apoptotic effect on the OSE that is very similar to that seen with OCs. This most interesting study is, however, difficult to interpret, because the progestin component alone would also block ovulation and follicle development, so that one cannot distinguish a direct progestin effect from an effect of suppression of follicle development and ovulation. This could be studied by using a GnRH analog to suppress ovulation.

What is the current situation regarding chemopreventive strategies against ovarian cancer? Oral contraceptive use remains an effective approach: in our data and in recently published studies, the protective effect was seen to be very long term, extending to women aged >60 years who had used OCs many years in the past. If the finding of a major protective effect of a late birth can be confirmed, then we may be able to exploit this for a short-term chemoprevention strategy with a long-term preventive effect. To do this, we will in all probability need to understand the mechanism of the protective effect. Because late incomplete pregnancies and late OC use do not appear to provide such an effect, we must look for the distinctive characteristics of a full-term pregnancy.

In vitro experiments on the effects of pregnancy levels of E and progesterone on ovarian tumor cells may be most informative. It is possible that the prolonged high levels of progesterone during a term pregnancy will be lethal to or will "terminally differentiate" a significant proportion of normal or "premalignant" ovarian cancer precursor cells. Observations on the ovaries of women immediately after delivery would be most informative, and this possibility could also be studied in macaques in a manner similar to that employed by Rodriguez et al. (45, 46) to study the effect of OCs.

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